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Bilateral ureteral obstruction alters levels of the G-protein subunits $G_{\alpha s}$ and $G_{\alpha q/11}$

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Bilateral ureteral obstruction alters levels of the G-protein subunits $G_{\alpha s}$ and $G_{\alpha q/11}$. To evaluate the effects of bilateral ureteral obstruction (BUO) on the levels of G-protein subunits in glomeruli, we examined the types and amounts of G-protein subunits in glomerular membranes from sham-operated control (SOC) rats and rats with BUO of 24 hours duration utilizing bacterial toxin-catalyzed ADP-ribosylation and specific antibodies. ADP-ribosylation catalyzed by cholera or pertussis toxin demonstrated the presence of G_s and G_i proteins in glomerular membranes. Immunoblots further revealed the existence of two types of $G_{\alpha s}$ (45 and 52 kDa), as well as $G_{\alpha i2}$ (40 kDa), $G_{\alpha i3}$ (41 kDa), $G_{\alpha q/11}$ (42 kDa) and G_β (35 to 36 kDa) in glomerular membranes. The predominant subspecies of $G_{\alpha s}$ was the 52 kDa protein. Detectable amounts of $G_{\alpha o}$ were not found in glomerular membranes. Moreover, G-protein subunits were not detected in cytosolic extracts of glomeruli. Both forms of $G_{\alpha s}$ and $G_{\alpha q/11}$ were significantly reduced in glomerular membranes from rats with BUO when compared to SOC rats. No significant difference in total $G_{\alpha i}$, $G_{\alpha i2}$ and $G_{\alpha i3}$ and G_β content was observed between the two groups of rats. *In vivo* pretreatment of rats with simultaneous administration of the angiotensin-converting enzyme inhibitor, enalaprilat, and the thromboxane synthase inhibitor, OKY-046, maintained the amount of $G_{\alpha s}$ and $G_{\alpha q/11}$ in rats with BUO at the levels seen in SOC rats. The two drugs did not affect the amounts of G-protein subunits in glomerular membranes of SOC rats. We conclude that the decreased mass of two forms of $G_{\alpha s}$ and $G_{\alpha q/11}$ in glomerular membranes from rats with BUO of 24 hours duration is caused by elevated levels of angiotensin II, thromboxane A_2, or both, that occur as a consequence of obstruction. This decrease in $G_{\alpha s}$ and $G_{\alpha q/11}$ content may contribute in part to the changes in glomerular function present at 24 hours after the onset of bilateral ureteral obstruction.

Guanine nucleotide-binding regulatory proteins (G-proteins) are a family of receptor-associated signal transducing proteins [1–4]. G-proteins are heterotrimers composed of α, β and γ subunits located in cell membranes. The diverse G-protein α subunits, which bind GTP and possess GTPase activity, are specific to each G-protein, while β and γ subunits have limited heterogeneity and are functionally interchangeable among several α subunits [3]. The α subunits of several G-proteins are substrates for ADP-ribosylation catalyzed by cholera or pertussis toxin. Historically this circumstance has been used to characterize specific G-protein families such as G_s, G_i and G_o [1–4]. More recently, several families of G-protein α subunits have been discovered by molecular cloning which are not derivitized by known bacterial toxins [1–4]. G-proteins dissociate into α and $\beta\gamma$ subunits by ligand-induced stimulation of receptors [1–4]. The dissociated α and $\beta\gamma$ subunits of G-proteins are active forms which regulate membrane-bound effectors such as phospholipases (A_2 and C), adenylate cyclase and ion channels [1–4]. The free $\beta\gamma$ subunits are tightly associated and serve as deactivators of α subunits by reforming the heterotrimer [1–4]. Recently, the relative distribution of G-proteins in different regions of the kidney has been reported [5–8].

Ureteral obstruction causes a progressive fall in glomerular filtration rate (GFR) and renal plasma flow [9–11]. These functional changes result from an increase in the activity of the vasoconstrictors thromboxane A_2 and angiotensin II, with a secondary rise in glomerular production of the vasodilatory eicosanoids, prostaglandin E_2 (PGE_2) and prostacyclin [12, 13]. The increased glomerular synthesis of PGE_2 and prostacyclin in the obstructed kidney may prevent a further fall in GFR and renal plasma flow by antagonizing the vasoconstrictive effects of elevated levels of angiotensin II and thromboxane A_2 after the onset of obstruction [9].

We have recently elucidated the mechanisms underlying the greater production of eicosanoids by glomeruli from rats with bilateral ureteral obstruction (BUO) of 24 hours duration [14]. Bilateral ureteral ligation of 24 hours duration increased the activities of both phosphatidylethanolamine-specific phospholipase A_2 and cyclooxygenase in glomeruli. Both of these enzymes have a key role in arachidonic acid metabolism. Phosphatidylcholine-specific phospholipase A_2 and phosphatidylinositol-4,5-biphosphate-specific phospholipase C did not appear to be involved in the greater synthesis of glomerular eicosanoids at 24 hours of BUO. The phospholipase activities designated as specific in actuality reflect preferential hydrolysis of these phospholipids in the presence of others. The preferential hydrolysis is designated as specificities in this paper. The present study shows that bilateral ureteral ligation causes alterations in the mass of some G-protein subunits in rat glomerular membranes by 24 hours after the onset of obstruction. G-protein subunits may have a role in the regulation of the activity of adenylate cyclase and phospholipases. The activities of both of these enzymes are affected following ureteral obstruction.

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Methods

Chemicals and reagents

Cholera toxin (CT), pertussis toxin (PT), thymidine, adenosine 5′-triphosphate (ATP), α-nicotinamide adenine dinucleotide (NAD), and goat anti-rabbit IgG (whole molecule) were obtained from Sigma (St. Louis, Missouri, USA). Rabbit antisera against $G_{\alpha i3}$ (EC/2) and $G_{\alpha o}$ (GC/2) were supplied from DuPont (Boston, Massachusetts, USA). ^{125}I sodium iodine was purchased from Amersham (Arlington Heights, Illinois, USA) and (adenylate-^{32}P)NAD was obtained from ICN (Irvine, California, USA).

Animal models and preparation of isolated glomeruli

Female Sprague-Dawley rats weighing approximately 200 g (Sasco, Inc., Omaha, Nebraska, USA) were utilized in the present study. The animals were divided into four groups according to the protocol previously reported [14]. Briefly, one half of the sham-operated control rats (SOC) and one half of the rats with bilateral ureteral obstruction (BUO) received five intraperitoneal (i.p.) injections of the angiotensin-converting enzyme inhibitor, enalaprilat (5 mg/kg), and the thromboxane synthase inhibitor, OKY-046 (20 mg/kg), dissolved in saline twice daily starting 48 hours prior to sham operation or obstruction. Surgery was performed one hour after the last i.p. injection of the two drugs. Under ether anesthesia rats were subjected to sham operation or BUO as previously reported [12–14]. After surgery the animals were given neither food nor water.

Isolated glomeruli were prepared as previously described from our laboratory [12–14]. Under pentobarbital anesthesia (5 mg/100 g body wt i.p.) 24 hours after sham surgery or bilateral ureteral ligation the abdominal cavity of each animal was opened, and both kidneys were thoroughly perfused with phosphate-buffered saline (PBS). The kidneys were immediately removed and decapsulated. The cortices were dissected on ice and glomeruli were obtained by sieve techniques (mesh size 250, 150 and 75 μm). The preparations consisted of more than 90% glomeruli.

Preparation of membranes and cytosolic extracts from isolated glomeruli

Glomerular membranes and cytosolic extracts were prepared as previously reported [14]. Isolated glomeruli were washed twice with cold Ca^{++}- and Mg^{++}-free Hanks' balanced salt solution (HBSS) by centrifugation/resuspension and suspended in 250 μl of ice-cold homogenate buffer [25 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol (DTT) and 1 KI unit/ml trazylol]. Glomerular homogenates were prepared with 10 strokes of a 0.7 ml suspension using a teflon-glass homogenizer and centrifuged at 48,000 × g for 20 minutes. The supernatants were stored at −70°C as cytosolic fractions. The pellets were washed with 2 ml of ice-cold homogenate buffer and recentrifuged at 48,000 × g for 20 minutes. The washed pellets were resuspended in 250 μl of ice-cold homogenate buffer and stored at −70°C as membrane fractions.

Toxin-catalyzed ADP-ribosylation and quantitation of guanine nucleotide regulatory proteins

ADP-ribosylation catalyzed by cholera toxin (CT) or pertussis toxin (PT) was performed in membranes of glomeruli using (adenylate-^{32}P) NAD as previously reported from our laboratory [15]. Briefly, glomerular membranes (approximately 50 μg protein) in homogenate buffer were incubated for 60 minutes at 37°C in 100 μl of 50 mM Tris chloride buffer, pH 7.5, containing 5 mM $MgCl_2$, 4 mM ATP, 40 mM thymidine, 0.1 mM GTP, 10 μM (^{32}P) NAD (10 μCi) and 10 μg of dithiothreitol (DTT)-preactivated CT or in 100 μl of 25 mM Tris-HCl buffer, pH 7.5, containing 5 mM $MgCl_2$, 4 mM ATP, 40 mM thymidine, 10 μM (^{32}P)NAD (10 μCi) and 1 μg of DTT-preactivated PT. The membrane preparations not incubated with cholera or pertussis toxin were utilized as non-toxin controls. The reaction was terminated by adding 2 ml of 10% trichloroacetic acid (TCA), and each sample was kept on ice for 60 minutes. Membrane proteins were collected by centrifugation at 3,000 rpm for 15 minutes and washed twice with 2 ml of diethylether to remove the TCA. The protein precipitate was dissolved in SDS sample buffer and heated for two minutes at 90°C. The membrane protein (15 μg protein) was subjected to 13.5% sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli [16]. The gel was stained-destained, dried and autoradiographed for 48 hours. The portion of the gel corresponding to $G_{\alpha s}$ (CT substrates) or $G_{\alpha i}$ (PT substrates) was cut out along with the corresponding segment of the non-toxin control (Fig. 1). The (^{32}P) activity was measured by liquid scintillation spectroscopy. Absolute amounts of $G_{\alpha s}$ or $G_{\alpha i}$ [fmole (^{32}P) per 15 μg of membrane protein] were calculated by subtracting the amount of radioactivity in the nontoxin control slice. $G_{\alpha s}$ and $G_{\alpha i}$ were completely ADP-ribosylated by CT and PT, respectively, after 60 minutes of incubation. This was determined from preliminary studies which entailed a time course of ribosylation.

Preparation of antisera and ^{125}I-labeled IgG

The peptides CRMHLRQYELL, representing amino acids 385–394 of the 52 kDa form of $G_{\alpha s}$-protein [17], CTVSAEDKAAAERSK representing amino acids 3–17 of $G_{\alpha i2}$-protein [18], CQLNLKEYNLV, representing amino acids 350–359 of $G_{\alpha 11}$ [19], and CMSELDQLRQE, representing amino acids 1–10 of G_{β} protein [20], were synthesized in our laboratory with the DuPont RAMPs system using the chemical protocols outlined by DuPont. The peptides were conjugated to keyhole limpet hemocyamine through the cysteine added to the amino terminus of each peptide. Each conjugate, emulsified with Freund's complete adjuvant, was subcutaneously injected into rabbits and boosted at monthly intervals with Freund's incomplete adjuvant. The specificity of antisera against $G_{\alpha s}$, $G_{\alpha i2}$, $G_{\alpha q/11}$ or G_{β} was examined by immunoblotting (see below). The antisera against peptide CRMHLRQYELL, peptide CTVSAEDKAAAERSK, peptide CQLNLKEYNLV, and peptide CMSELDQLRQE specifically recognized the 45 and 52 kDa ($G_{\alpha s}$), 40 kDa ($G_{\alpha i2}$), 42 kDa ($G_{\alpha q/11}$) and 35 to 36 kDa (G_{β}) G-proteins, respectively (Figs. 2 and 3). The specificity of these antibodies was further demonstrated by Western blotting using bacterial lysates containing recombinant $G_{\alpha s}$ (short), $G_{\alpha i1}$, $G_{\alpha i2}$, $G_{\alpha i3}$ and $G_{\alpha o}$ (data not shown). These lysates were provided by

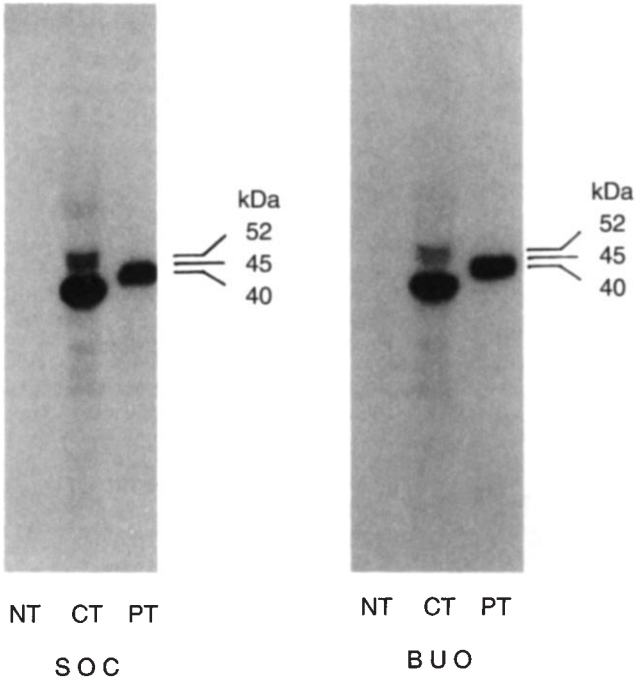

Fig. 1. *Autoradiographic replicas of cholera ($G_{\alpha s}$) and pertussis ($G_{\alpha i}$) toxin substrates ADP-ribosylated in glomerular membranes from sham-operated control rats (SOC) and rats with bilateral ureteral obstruction (BUO).* Glomerular membranes were incubated at 37°C for 60 minutes with (^{32}P) NAD in the presence of either cholera toxin (CT) or pertussis toxin (PT). Membrane preparations not treated with CT or PT were used as non-toxin (NT) controls. The membrane proteins (15 μg) were subjected to SDS-PAGE and the gel was stained-destained, dried and autoradiographed for 48 hours. The molecular weights were determined from the migration of standard proteins.

Dr. Juan Codina (Baylor College of Medicine, Houston, Texas, USA). The detailed characterization of these antibodies has been recently described [6]. The antisera to $G_{\alpha q/11}$ did not recognize any protein of the bacterial lysates but did specifically react with the immunizing $G_{\alpha q/11}$ peptide by ELISA (Fig. 4). There was no significant reactivity with other G-protein peptides. In addition, the antisera GC/2 [21] specifically recognized $G_{\alpha o}$ (39 kDa) proteins. The antisera EC/2 [22] specifically reacted with $G_{\alpha i3}$ (41 kDa) proteins with a minimal recognition of $G_{\alpha o}$ proteins.

Goat anti-rabbit IgG was radiolabeled with ^{125}I by the chloramine T method. The ^{125}I-labeled IgG was separated from unreacted radioiodine by passage over a G-50 column (size, 5 × 200 mm) equilibrated with 50 mM phosphate buffer, pH 7.0. Goat anti-rabbit IgG labeled with ^{125}I were used as second antibodies of immunoblots for the determination of G-protein subunits ($G_{\alpha s}$, $G_{\alpha i2}$, $G_{\alpha i3}$, $G_{\alpha o}$, $G_{\alpha q/11}$ and G_{β}).

Immunoblotting for the determination of G-protein subunits

Immunoblotting was performed to determine the levels of G-protein subunits $G_{\alpha s}$, $G_{\alpha i2}$, $G_{\alpha i3}$, $G_{\alpha o}$, $G_{\alpha q/11}$ and G_{β} in glomeruli. Membranes or cytosolic extracts of glomeruli were dissolved in SDS sample buffer and heated for two minutes at 90°C. The samples (10 μg protein) were subjected to SDS-PAGE. The proteins were electrophoretically transferred to nitrocellulose membranes in cold transfer buffer (20% methanol containing 25 mM Tris and 190 mM glycine). The nitrocellulose membrane was washed three times for five minutes with 20 mM Tris-HCl, pH 7.5, containing 500 mM NaCl (A-buffer) to remove methanol and then incubated with 20 mM Tris-HCl, pH 7.5, containing 3% gelatin, 500 mM NaCl and 0.02% NaN_3 to saturate non-specific protein binding sites. After washing three times for five minutes each with 20 mM Tris-HCl, pH 7.5, containing 500 mM NaCl and 0.05% tween 20 (B-buffer), the membrane was incubated for one hour at room temperature with rabbit antisera against $G_{\alpha s}$ (1:200 dilution), $G_{\alpha i2}$ (1:100 dilution), $G_{\alpha i3}$ (1:1000 dilution), $G_{\alpha o}$ (1:1000 dilution), $G_{\alpha q/11}$ (1:200 dilution), or G_{β} (1:100 dilution) in immunoblotting buffer (20 mM Tris-HCl, pH 7.5, containing 500 mM NaCl, 1% gelatin, 0.5% tween 20 and 0.02% NaN_3). The nitrocellulose sheets were then washed three times in B-buffer before being immersed in immunoblotting buffer containing ^{125}I-labeled goat anti-rabbit antibodies (4 million cpm/ml) for 30 minutes at room temperature, washed and autoradiographed for 24 hours. The autoradiographs were scanned with a GS 300 Densitometer (Hoefer Scientific Instruments, San Francisco, California, USA). Values of each band are in arbitrary units (Fig. 3). All samples were run simultaneously to determine each protein and to eliminate interassay variation, which contributes to the consistency of the determinations.

Calculations and statistical analysis

Each glomerular preparation was obtained from one or two rats and all data reported represent the mean ± SE of five separate glomerular preparations. The protein content of glomerular preparations was determined by the method of Lowry et al [23]. Statistical analysis was performed by Student's *t*-test.

Results

Determination and quantitation of guanine nucleotide regulatory proteins by ADP-ribosylation of glomerular membranes

G-proteins which were ADP-ribosylated by cholera toxin (CT) or pertussis toxin (PT) were examined to elucidate the types and absolute amounts of guanine nucleotide regulatory proteins (G-proteins) in glomerular membranes. Figure 1 shows an autoradiographic replica obtained from CT- or PT-catalyzed ADP-ribosylation of glomerular membranes from SOC rats and rats with BUO. Significant amounts of CT-sensitive (45 and 52 kDa) and PT-sensitive (40 to 41 kDa) G-protein α subunits, $G_{\alpha s}$ and $G_{\alpha i}$, were present in glomerular membranes. The CT-sensitive and PT-sensitive substrates were not detected in cytosolic extracts of glomeruli (data not shown). In the CT-catalyzed ADP-ribosylation an intense autoradiographic band of lower molecular weight appeared to be unidentified CT-sensitive substrates, because specific $G_{\alpha s}$ and $G_{\alpha o}$ antibodies did not recognize the proteins in this area (Fig. 2). This indicates that at least two families of G-proteins, $G_{\alpha s}$ and $G_{\alpha i/\alpha o}$, are present in glomerular membranes. Since immunoblots (see below) did not detect $G_{\alpha o}$ the PT-sensitive substrates are of the $G_{\alpha i}$ family.

Table 1 presents data on the absolute amounts of $G_{\alpha s}$ and $G_{\alpha i}$ calculated from ADP-ribosylation of glomerular membranes from SOC rats and rats with BUO. The absolute amounts of $G_{\alpha s}$ and $G_{\alpha i}$ were determined in five separate glomerular membrane

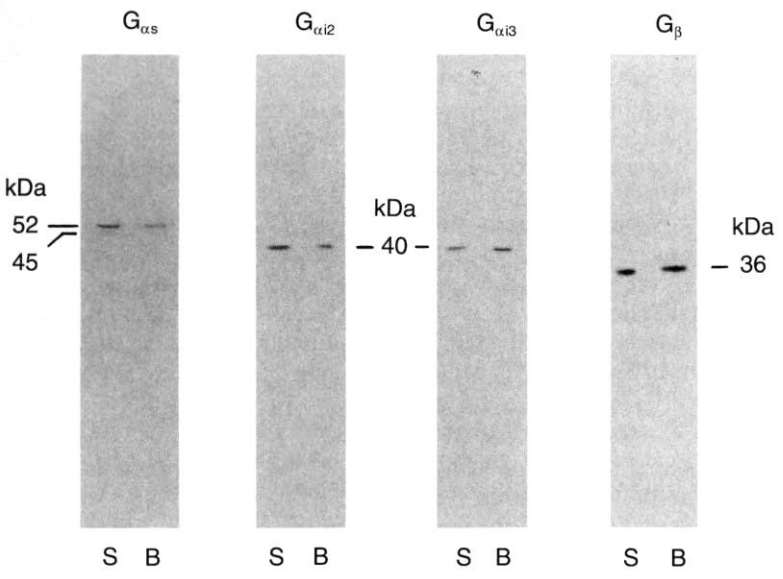

Fig. 2. *Autoradiographic replicas of immunoblots for $G_{\alpha s}$, $G_{\alpha i2}$, $G_{\alpha i3}$ and G_{β} in glomerular membranes (10 μg protein) from sham-operated control rats (S) and rats with bilateral ureteral obstruction (B).* Glomerular membrane proteins were subjected to SDS-PAGE and transferred to a nitrocellulose membrane. The nitrocellulose was incubated for 60 min with antibodies against $G_{\alpha s}$, $G_{\alpha i2}$, $G_{\alpha i3}$ or G_{β} in immunoblotting buffer. The membrane was further incubated for 30 min with ^{125}I-labeled second antibodies in immunoblotting buffer and subsequently washed, dried and autoradiographed for 24 hours.

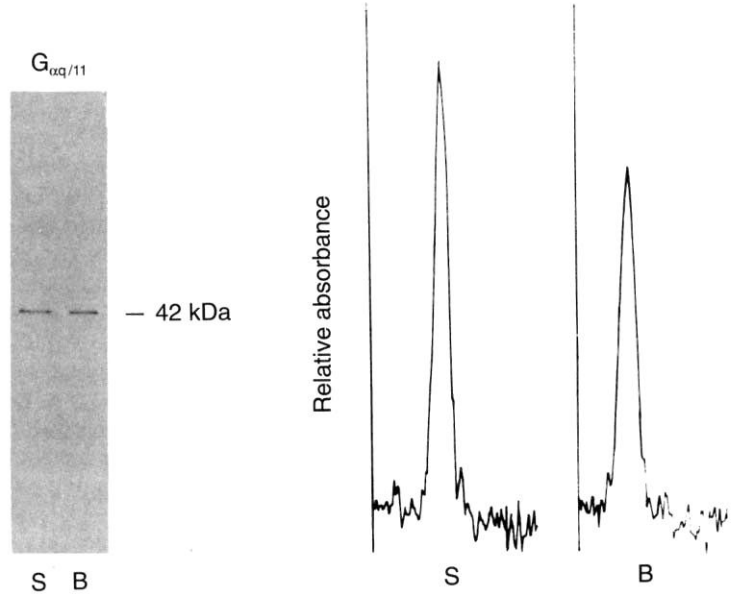

Fig. 3. *Autoradiographic replica and densitometry tracing of an immunoblot for $G_{\alpha q/11}$ in glomerular membranes (10 μg protein) from sham-operated control rats (S) and rats with bilateral ureteral obstruction (B).* See Figure 2 for details except for the use of antibody against $G_{\alpha q/11}$.

preparations obtained from each group of animals. $G_{\alpha s}$ content was reduced by 33% in glomerular membranes of rats with BUO when compared to SOC rats ($P < 0.005$). No significant difference in $G_{\alpha i}$ content was observed between the two groups. There was no consistent change in the amount of the lower molecular weight unidentified CT substrate.

Levels of $G_{\alpha s}$, $G_{\alpha i2}$, $G_{\alpha i3}$, $G_{\alpha q/11}$ and G_{β} in glomerular membranes

Figure 2 shows an autoradiographic replica obtained from immunoblots for the determination of G-protein subunits ($G_{\alpha s}$, $G_{\alpha i2}$, $G_{\alpha i3}$ and G_{β}) in glomerular membranes from SOC rats and rats with BUO. Figure 3 shows an autoradiographic replica obtained from immunoblots for the determination of G-protein subunits $G_{\alpha q/11}$, along with a densitometer scan. This antibody was obtained after the other antibodies; hence the separate figures. Immunoblots confirmed the presence of two forms of $G_{\alpha s}$ (45 and 52 kDa) which were detected by ADP-ribosylation (Fig. 1) in glomerular membranes. Immunoblots further demonstrated the existence of $G_{\alpha i2}$ (40 kDa), $G_{\alpha i3}$ (41 kDa), $G_{\alpha q/11}$ (42 kDa) and G_{β} (35 to 36 kDa) in glomerular membranes [24, 25]. However, $G_{\alpha o}$ was not detectable in significant amounts. As with ADP-ribosylation, immunoblotting revealed no detectable amounts of $G_{\alpha s}$, $G_{\alpha i2}$, $G_{\alpha i3}$, $G_{\alpha o}$, $G_{\alpha q/11}$ and G_{β} in cytosolic extracts of glomeruli (data not shown).

Table 2 presents data on the relative levels of $G_{\alpha s}$, $G_{\alpha i2}$, $G_{\alpha i3}$, $G_{\alpha q/11}$ and G_{β} in glomerular membranes obtained from SOC rats, and rats with BUO treated or not treated with enalaprilat and OKY-046 prior to sham operation or bilateral ureteral ligation. The predominant subspecies of $G_{\alpha s}$ was a 52 kDa protein in glomerular membranes of both SOC rats and rats with BUO. When animals were not pretreated with OKY-046 and

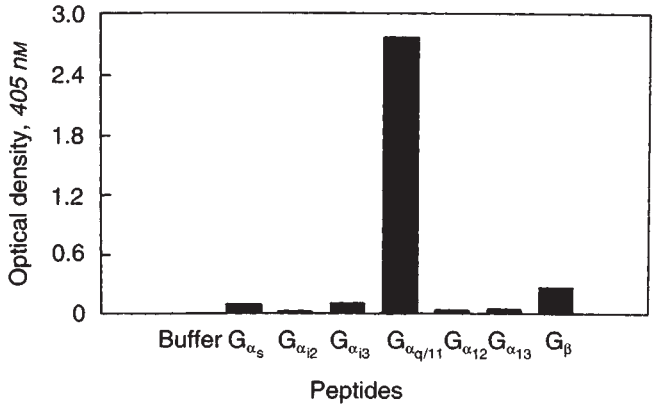

Fig. 4. *ELIZA analysis of the specificity of the $G_{\alpha q/11}$ antibody.* Microtiter plate wells were coated with the different G-protein peptides used to elicit antibodies. A 1/500 dilution of an antibody developed to the $G_{\alpha q/11}$ peptide was allowed to react with the peptides. A goat antirabbit IgG linked to alkaline phosphatase was used to quantitate the primary antibody.

Table 1. Quantitation of G-proteins in glomerular membranes from sham-operated control rats (SOC) and rats with bilateral ureteral obstruction (BUO)

	$G_{\alpha s}$ (Cholera)	$G_{\alpha i}$ (Pertussis)
	fmol [^{32}P]ADP-ribose incorporated/ 15 μg protein	
SOC	9.3 ± 0.2	12.2 ± 2.0
BUO	6.2 ± 0.5[a]	11.6 ± 1.5

Quantities of $G_{\alpha s}$ and $G_{\alpha i}$ proteins were determined from the amount of [^{32}P]ADP-ribosylation by cholera and pertussis toxin as described in **Methods**. Data are reported as fmol [^{32}P]ADP-ribose incorporated/15 μg glomerular membrane protein. Values are means ± SE obtained from five separate glomerular preparations. Abbreviations are: G-proteins, guanine nucleotide-binding regulatory proteins: $G_{\alpha s}$, stimulatory G-protein α-subunits; $G_{\alpha i}$, inhibitory G-protein α-subunits; SOC, sham-operated control; BUO, bilateral ureteral obstruction.

[a] $P < 0.005$ compared to the SOC value

enalaprilat, the amounts of 45 kDa and 52 kDa proteins of $G_{\alpha s}$ were significantly decreased—by 34% ($P < 0.005$) and 24% ($P < 0.005$), respectively—in glomerular membranes from rats with BUO, when compared to SOC rats. Therefore, the total $G_{\alpha s}$ content was substantially reduced, by 27% ($P < 0.005$), in glomerular membranes of rats with BUO when compared to glomerular membranes of SOC rats. The reduction was comparable to that seen with ADP-ribosylation (Table 1). There was also a significant decrease (23%) in the levels of $G_{\alpha q/11}$ in glomerular membranes of BUO rats. The decreased amount of $G_{\alpha s}$ and $G_{\alpha q/11}$ in glomerular membranes of rats with BUO, however, was not seen when the rats were pretreated with enalaprilat and OKY-046. By contrast, there was no significant difference in the total $G_{\alpha i}$ content measured by pertussis toxin. Immunoblots revealed no change in $G_{\alpha i2}$ and $G_{\alpha i3}$, and no decrease in one balancing an increase in the other. Furthermore, G_{β} content of glomeruli did not differ among the four groups of rats (Table 2).

Discussion

In the present study ADP-ribosylation catalyzed by bacterial toxins clearly demonstrated that G-protein families, G_s and G_i,

Table 2. Relative levels of G-protein subunits in glomerular membranes from sham-operated control (SOC) rats and rats with bilateral ureteral obstruction (BUO) pretreated or not with enalaprilat and OKY-046 prior to sham operation or obstruction

	Rats not given enalaprilat and OKY-046		Rats given enalaprilat and OKY-046	
	SOC	BUO	SOC	BUO
$G_{\alpha s}$				
45 kDa	5.6 ± 0.3	3.7 ± 0.3[a]	5.3 ± 0.1	5.7 ± 0.4
52 kDa	11.7 ± 0.5	9.0 ± 0.2[a]	12.3 ± 0.6	12.3 ± 0.4
Total	17.3 ± 0.8	12.7 ± 0.3[a]	17.6 ± 0.6	18.0 ± 0.4
$G_{\alpha i2}$	7.0 ± 0.3	7.3 ± 0.3	7.1 ± 0.4	7.3 ± 0.6
$G_{\alpha i3}$	12.5 ± 0.4	11.5 ± 0.6	11.6 ± 0.5	11.6 ± 0.5
$G_{\alpha q/11}$	15.8 ± 0.4	12.2 ± 0.4[a]	16.0 ± 0.3	15.6 ± 0.2
G_{β}	44.0 ± 0.8	46.6 ± 1.9	46.0 ± 1.6	46.5 ± 1.5

Glomerular membranes were prepared from SOC and BUO rats that did not or did receive 5 mg/kg enalaprilat and 20 mg/kg OKY-046 prior to sham operation or obstruction. Relative levels of G-protein subunits in glomerular membranes were determined by scanning the blots obtained from autoradiography of immunoblots with a densitometer as described in **Methods**. Data reported are in arbitrary units per 10 μg of membrane protein. Values are means ± SE obtained from five separate glomerular preparations. Abbreviations are: G-protein, guanine nucleotide-binding regulatory protein; $G_{\alpha s}$, stimulatory G-protein α-subunits; $G_{\alpha i2}$, inhibitory G-protein αi2-subunits; $G_{\alpha i3}$, inhibitory G-protein αi3-subunits; $G_{\alpha q/11}$, bacterial toxin-insensitive G-protein αq and α11 subunits; G_{β}, G-protein β-subunits; SOC, sham-operated control; BUO, bilateral ureteral obstruction.

[a] $P < 0.005$ compared to each SOC value

are present in glomerular membranes. Immunoblotting with specific antibodies revealed the presence of two different $G_{\alpha i}$ subunits, $G_{\alpha i2}$ and $G_{\alpha i3}$ in glomerular membranes. Immunoblotting further demonstrated the existence of the bacterial toxin-insensitive G-protein alpha subunits q/11. Preliminary results of homology-based PCR cloning experiments revealed the existence of $G_{\alpha 11}$, but not $G_{\alpha q}$, in rat glomeruli (data not shown). Our antibody recognizes both proteins due to their common carboxy terminal sequence, used to immunize the rabbits. Also, immunoblots showed that $G_{\alpha s}$ in glomerular membranes had two forms, a 52 kDa protein and a lesser amount of a 45 kDa protein. In contrast to glomerular membranes, brush border and basolateral membranes from rat renal cortex have been found to contain a 45 kDa protein as the predominant subspecies of $G_{\alpha s}$ subunit [6]. It is recognized that the glomerulus is composed of endothelial, epithelial and mesangial cells and is, therefore, quite heterogeneous in cell type. These studies, therefore, are a measure of the G-protein content integrated over the whole glomerulus. Further studies will be needed to define which cell type(s) have a reduction in $G_{\alpha s}$ and $G_{\alpha q/11}$ due to ureteral obstruction.

We have most recently reported the heterogeneous distribution of several G-protein subunits, $G_{\alpha s}$, $G_{\alpha i2}$, $G_{\alpha i3}$, and G_{β} in rat renal nephron segments [6], an observation similar to that reported simultaneously by Stow, Sabolic and Brown [8]. Immunocytochemical studies demonstrated the presence of $G_{\alpha s}$, $G_{\alpha i3}$ and G_{β} subunits in glomeruli [6, 8]. However, no immunofluorescence staining was observed for $G_{\alpha i2}$ proteins in glomeruli [6, 8]. This may be because the content of $G_{\alpha i2}$ subunits in glomeruli is below the limit of detection by immunocytochemical means. Indeed, in the present study Western blot analysis detected significant amounts of $G_{\alpha i2}$ in glomerular

membranes when purified and concentrated membrane preparations of glomeruli were utilized. Moreover, Northern blot analysis revealed detectable amounts of mRNA for $G_{\alpha i2}$ and $G_{\alpha i3}$ in rat glomerular mesangial cells [7]. However, no message for the expression of $G_{\alpha i1}$ mRNA was detected in mesangial cells [7]. Our previous immunocytochemical study was essentially completed prior to our preparation of antibodies which specifically recognized $G_{\alpha q/11}$.

Of importance in the present study was the finding that bilateral ureteral ligation of 24 hours duration caused a significant decrease in two forms of $G_{\alpha s}$ and in $G_{\alpha q/11}$ of glomerular membranes. By contrast, the total $G_{\alpha i}$, $G_{\alpha i2}$ and $G_{\alpha i3}$, and G_{β} content was essentially similar in glomerular membranes of SOC and BUO rats. Due to the heterogeneous nature of the glomerulus it is possible that these G-protein subunits could have decreased in one cell type(s) but had been coincidentally increased in another cell type(s). This awaits further study. The decrease in $G_{\alpha s}$ and $G_{\alpha q/11}$ associated with BUO may be due to the elevated levels of angiotensin II, thromboxane A_2, or both vasoconstrictors, that occurs after the onset of obstruction. Indeed, pretreatment of the rats with the angiotensin-converting enzyme inhibitor, enalaprilat, and the thromboxane synthase inhibitor, OKY-046, maintained the levels of $G_{\alpha s}$ and $G_{\alpha q/11}$ in glomerular membranes of BUO rats at levels comparable to those observed in membrane preparations of SOC rats.

A role of G-proteins in the regulation of membrane-linked effectors has been elucidated [1–4, 26]. $G_{\alpha s}$ activates adenylate cyclase [1–4] and angiotensin II decreases cyclic AMP levels on a short-term basis [27]. Schlondorff et al [28] found that adenylate cyclase activities were significantly lower, by 51%, in glomeruli isolated from the ligated kidney than in glomeruli from the contralateral kidney of rats with unilateral ureteral obstruction of 72 hours duration. This may indicate that there is a long-term inhibition of cyclic AMP production by endogenous angiotensin II through a decrease in $G_{\alpha s}$ content. The effects of thromboxane A_2 on the activity of adenylate cyclase in glomeruli have not been well characterized. $G_{\alpha i2}$ may stimulate membrane-linked phospholipase C in rat glomerular mesangial cells in culture [7]. There is strong evidence for a role of $G_{\alpha q/11}$ in the stimulation of phospholipase C-β_1 activity [26]. In a previous study we found that phosphatidylinositol-4,5-biphosphate (PIP_2)-specific PLC activities were decreased in glomeruli of rats with BUO compared to SOC rats [14]. In the present study the amounts of $G_{\alpha i2}$ in glomerular membranes appear to be comparable in the two groups of rats. These differences could be attributable to the systems used, that is, cultured mesangial cells [7] versus freshly isolated glomeruli in the present study. A real difference was the decreased amount of $G_{\alpha q/11}$ found in the glomerular membranes of BUO rats. This factor alone could account for the decrease in PIP_2-specific phospholipase C activity of the glomerular membranes of BUO animals [14]. This assertion awaits characterization of the type of phospholipase C isoenzyme(s) that is(are) reduced in the glomerulus of obstructed kidneys.

Decreased amounts of $G_{\alpha s}$ and $G_{\alpha q/11}$ with no changes in the $G_{\alpha i2}$, $G_{\alpha i3}$, and G_{β} content may cause an increase in the relative levels of free $\alpha\gamma$ subunits in glomerular membranes of rats with BUO of 24 hours duration. The free $\beta\gamma$ subunits, which are active forms of the G-protein subunits, have been shown to stimulate membrane-bound phospholipase A_2 in rat heart and bovine retina [29–31]. Indeed, phosphatidylethanolamine (PE)-specific phospholipase A_2 activities were significantly elevated in glomerular membranes from rats with BUO when compared to SOC rats [14]. Moreover, in the previous and present study the increased activities of PE-specific phospholipase A_2 [14] and the decreased $G_{\alpha s}$ and $G_{\alpha q/11}$ content in glomerular membranes of rats with BUO were not seen in animals treated with enalaprilat and OKY-046 prior to obstruction. Administration of the two drugs to SOC rats did not affect the amounts of G-protein subunits and the activities of phospholipases [14] in glomeruli. These results suggest, but do not prove, a potential role of free $\beta\gamma$ subunits in the activation of PE-specific phospholipase A_2 in glomerular membranes of rats with BUO. To evaluate this hypothesis, further studies on direct stimulation of phospholipase A_2 in glomeruli by $\beta\gamma$ subunits purified from glomerular membranes need to be performed.

In summary, several G-protein subunits, $G_{\alpha s}$, $G_{\alpha i2}$, $G_{\alpha i3}$, $G_{\alpha q/11}$ and G_{β}, are present in rat glomerular membranes. Bilateral ureteral ligation caused a modest but significant decrease in the amounts of two forms of $G_{\alpha s}$ and in $G_{\alpha q/11}$, but no changes in total $G_{\alpha i}$, $G_{\alpha i2}$, $G_{\alpha i3}$ and G_{β} content. Levels of $G_{\alpha s}$ and $G_{\alpha q/11}$ in glomerular membranes of rats with BUO remained at the levels seen in membrane preparations of SOC rats when the rats were treated with enalaprilat and OKY-046 prior to obstruction. We conclude that BUO of 24 hours duration causes a decrease in the mass of two forms of $G_{\alpha s}$ and in $G_{\alpha q/11}$ through the elevated levels of angiotensin II, thromboxane A_2, or both, that occur as a consequence of obstruction. These decreased levels of $G_{\alpha s}$ and $G_{\alpha q/11}$ may contribute in part to the changes in glomerular function that are observed at 24 hours after the onset of BUO.

Note added in proof

Since acceptance of this manuscript, we have found that our antibody to $G_{\alpha q/11}$ also recognizes $G_{\alpha 14}$. This G-protein also stimulates phospholipase C activity.

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